revealed that neutrophil rolling on P-selectin at a shear stress of 6-8 dyn/cm^2 is facilitated by four-fold larger footprints than expected and three to four long membrane tethers at the rear. When mouse bone marrow neutrophils were stained with an intercalating membrane dye (DiI), allowed to roll on P- selectin in a microfluidic device at a shear stress of 6-10 dyn/cm^2 and footprints recorded using dual-color qDF (DqDF), we found that following tether bond failure, tethers did not retract. Instead, the detached tethers landed as 'slings' in front of the rolling neutrophils. Slings were confirmed in an acute model of inflammation *in vivo* using epifluorescence-intravital microscopy of mouse cremaster. In each sling, PSGL-1 was distributed in patches 1.6 μm apart while the integrin LFA-1 was expressed over the entire length of slings. As the cells rolled on slings, they were wrapped around the rolling neutrophils and underwent a step-wise peeling at the rear of the rolling neutrophils enabled by the tandem failure of PSGL-1 patches under hydrodynamic force. The failure of each load-bearing PSGL-1 patch on the sling resulted in loading of the next patch downstream of it and the cycle continued until the sling was completely peeled off from the substrate. 'Peeling of slings' is distinct from 'pulling of tethers' reported previously and the two mechanisms probably work synergistically to facilitate neutrophil rolling at high shear stress during inflammation. This study was supported by the American Heart Association-Scientist Development Grant 11SDG7340005 (P.S.) and NIH EB02185 (K.L.).

978-Pos Board B764

Label-Free Hyperspectral Imaging of Intracellular Hemoglobin in Human Erythrocytes

Ji Youn Lee[1], Fuyuki Tokumasu[2], Dan Sackett[2], Ralph Nossal[3], Jeeseong Hwang[1].
[1]National Institute of Standards and Technology, Gaithersburg, MD, USA, [2]National Institutes of Health, Bethesda, MD, USA, [3]National Institute of Health, Bethesda, MD, USA.

A broadband hyperspectral imaging (HSI) technique, originally developed for remote sensing and imaging in geophysical applications, recently has been employed to enable noninvasive label-free optical imaging of biological specimens. Here we report our results on hyperspectral microscopy of non-labeled erythrocytes to study the distribution of hemoglobin. We have chosen to focus on cellular hemoglobin because it is an endogenous chromorphore that is present in high concentrations, which makes it a good candidate for establishing proof of principle. In our imaging technique, hyperspectral data cubes (x, y, λ) are collected by either a wide-field or confocal microscope equipped with a broadband light source, such as a white light laser or spectral light engine in which narrow-band selection and wavelength scanning is achieved by fast optoelectronic devices. The obtained data cubes then are analyzed by algorithms based on a spectral angle mapper, yielding a set of unique absorption spectral signatures (i.e. endmembers) corresponding to subforms of hemoglobin, such as oxyhemoglobin and methemoglobin. Wavelength-dependent scattering signatures of cell membranes are also resolved. Unique endmembers of specific hemoglobin subforms are identified and used to build a map of intracellular hemoglobin distribution by estimating the abundance of each specific endmembers from the hyperspectral data cube. Our ongoing efforts include technology development for the determination of the local thickness of a single erythrocyte, to enable single cell volumetric measurement. The technique may be further developed for label-free molecular and chemical imaging of a broad range of endogenous biomarkers in cells and tissues, and ultimately for in vivo molecular imaging as well.

979-Pos Board B765

Quantifying Bacterial Growth and Gene Expression using New Long-Term Single-Cell Observation System

Mikihiro Hashimoto[1], Yuichi Wakamoto[2,3].
[1]Department of Basic Science, Graduate School of Arts and Sciences, The University of Tokyo, Tokyo, Japan, [2]Center for Complex Systems Biology, Graduate School of Arts and Sciences, The University of Tokyo, Tokyo, Japan, [3]JST PRESTO, Tokyo, Japan.

Although the phenotypic differences between individual organisms can often be ascribed to underlying genetic and environmental variation, even genetically identical organisms in homogeneous environments are phenotypically heterogeneous due to the stochastic effects in biological reactions, which is considered to play an important role in the biological processes such as differentiation, development, adaptation and evolution. In general, the single-cell distribution of the state of a cell (e.g., protein concentration) can be obtained by repeating a single-cell measurement over an ensemble of cells. However, we must be careful that of the result reflects not only the statistical property of individual cells, but also the difference of cell proliferation rate that depends on the temporary state. Conversely, a careful treatment of the statistical properties of cell lineage and population might allow a reliable detection of the relation between proliferation and the other phenotypic states. To address the question along this line, we asked how cell growth fluctuations and gene expression fluctuations are interrelated. For this purpose, we developed a new microfluidic device that allows rigorous environmental control and tracking of a large number of individual growing cells for hundreds of generations and designed a new custom software to analyze timelaps images using imageJ and C. Analysis of long-term data of individual histories obtained by our new system demonstrated the discrepancies of the growth rate as well as of the gene expression level distributions between the single-cell lineages and the population. We confirmed that a simple mathematical relation, which was in fact discovered more than 60 years ago, can explain the difference of growth rate. This study shows the significance of large-scale and careful examination of individual lineage trees, which provides the non-trivial statistical relations between individual cells and population.

980-Pos Board B766

3D Imaging by Multiphoton Selective Plane Illumination

Francesca Cella Zanacchi[1], Zeno Lavagnino[1,2], Emiliano Ronzitti[1], Giuseppe Vicidomini[1], Cesare Usai[3], Alberto Diaspro[1,2].
[1]Italian Institute of Technology (IIT), Genoa, Italy, [2]DIFI-University of Genoa, Genoa, Italy, [3]IBF. CNR, Genoa, Italy.

Light-sheet based techniques, such as single plane illumination microscopy (SPIM) [1] and digital scanned laser microscopy (DSLM) [2], have been found particularly useful in developmental biology applications since they provide the capability to perform fast imaging of living samples reducing photobleaching effects. In particular, single plane illumination provides high signal to noise ratio and optical sectioning capability due to the planar excitation, representing a useful tool for biological investigations of thick biological samples.
On the other side, two photon excitation microscopy (2PE) has been demonstrated to be a suitable tool for improving penetration depth since it allows for reduction of the scattering effects and light-sample interactions. To improve image quality and penetration depth of light sheet illumination microscopy, two photon excitation can be coupled with planar illumination [3] thus reducing the scattering effects due to light-sample interactions.
We characterized the two photon excitation process within the light sheet and we applied this technique to image large biological samples [4]. 3D imaging of mammary cell spheroids in depth has been performed using the resulting hybrid architecture.
Keywords: Two photon excitation, Single plane illumination microscopy.
(1) Huisken, J., et al. *Science* **305**, 1007-1009 (2004).
(2) Keller, P. J., Schmidt, A. D. , Wittbrodt, J., and Stelzer, E.H.K. Science, 322 (2008).
(3) Truong et al. *Nature Methods* 8,757-760, (2011)
(4) Cella Zanacchi et al. *Proc. of SPIE*, vol.7903 ''Multiphoton Microscopy in the Biomedical Sciences XI'' (2011)

981-Pos Board B767

Characterization of Scattering Effects in Phantom Samples using Single and Two-Photon Excitation Light Sheet Microscopy

Zeno Lavagnino[1,2], Francesca Cella Zanacchi[1], Emiliano Ronzitti[1], Ivan Coto Hernandez[1], Alberto Diaspro[1,2].
[1]Italian Institute of Technology, Genoa, Italy, [2]Department of Physics, University of Genoa, Genoa, Italy.

Scattering in thick biological samples plays a relevant role in imaging degradation and reduction of signal to noise ratio and contrast. Even if there are existing techniques that allow imaging of very thick samples, they can suffer from aberrations and distortions related to scattering properties of the sample. Recently, the capability of coupling light sheet techniques with Two Photon Excitation(TPE) (1,2,3) provided a powerful tool for deep imaging of biological samples. This has been proved to be a promising trend in thick-imaging microscopy, because it combines the advantages of light sheet techniques with the increased penetration depth intrinsic of TPE. For this reason, in this work a detailed characterization of the effects induced by scattering on the excitation profile of a light sheet based microscope is provided. In particular, different phantom samples, with different scattering coefficients mimicking the optical properties of various biological tissues, are used. Experiments were performed to investigate the shape distortions of the excitation intensity distribution provided by the light sheet, both in single and in two-photon excitation. This aspect represents a crucial point, since the effective light sheet intensity distribution is strictly related to the optical sectioning capability and image quality of the system. Results show that the light sheet intensity distribution is less affected by light-sample interactions and the shape is preserved in the case of TPE in comparison with single photon excitation regime. Furthermore, contrast measurements has been performed using fluorescent beads embedded in different